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Secondary conidiation of *Sphacelia sorghi* on sorghum, a novel factor in the epidemiology of ergot disease

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Sphacelia sorghi, the ergot pathogen of sorghum in Zimbabwe, causes copious exudation of honeydew containing macroconidia. Within a few days the exudate develops a white crust consisting of a layer of secondary conidia borne above the honeydew surface on a palisade of sterigma-like projecting hyphae which arise from the macroconidia immediately below the honeydew surface. Secondary conidia are windborne, initiate infection and are recognized for the first time to have an important role in the epidemiology of ergot disease of sorghum in Southern Africa.

Key words: Secondary conidia, Epidemiology, Ergot, Sorghum, Honeydew, *Sphacelia sorghi*.

The epidemiology of ergot diseases has been assumed to involve primary infections due to airborne ascospores, followed by secondary spread of the disease by direct dissemination of the sphacelial conidia released in honeydew from infected florets. Conidial dissemination in the field is, therefore, mainly by insects and other animal vectors, rain-splash and/or head to head contact between inflorescences. *Claviceps purpurea* (Fr.) Tul. is the model for these assumptions. It is well-known that epidemics of this pathogen are relatively localized and even commercial ergot cultivation on rye does not pose a significant threat to nearby cereal crops.

In the tropics *C. fusiformis* Loveless causes local epiphytotics of disease in pearl millet; the incidence of infected florets may be quite extensive on account of the pronounced protogyny of this crop plant but there has been no suggestion that the biology of the pathogen deviates from the *C. purpurea* model. Similarly, the effectively protogynous habit of male-sterile sorghum seemed to explain infection by *Sphacelia sorghi* McRae, its ergot parasite, when it was first seen to be prevalent in experimental sorghum in Nigeria in the 1960s (Futrell & Webster, 1966). More recently, however, increased planting of experimental sorghum in semi-arid parts of Southern and East Africa, and the use of hybrids, has been matched by ergot disease epiphytotics even when the crop is newly introduced into a locality. As, according to the literature, there are no obvious alternate hosts for *S. sorghi*, the origin of sorghum ergot epiphytotics has been a mystery, especially since the ascosporogenous stage, being so rare that it has not yet been fully described, is probably not a significant part of the life cycle of the fungus.

Extension of a study of sorghum ergot disease caused by an Indian pathogen (Frederickson & Mantle, 1988) to include African pathotypes has facilitated observation of contrasting natural mycological events which now add an important new dimension to the epidemiology of some ergot fungi.

MATERIALS AND METHODS

Experimental pathology

Male-sterile sorghum (IS 2219A, ICRISAT) was grown in a horticultural tunnel of conventional design in Surrey, U.K. (Frederickson & Mantle, 1988) to flower in Aug. 1987. Field plots of male-sterile and fertile sorghum cultivars were grown at Bulawayo, Zimbabwe in Feb. to Apr. 1988.

A spore suspension of *S. sorghi* indigenous to Zimbabwe was prepared from naturally infected sorghum and sprayed, with a hand atomizer, on sorghum inflorescences at floret gaping or anthesis. Care was taken to prevent drift of spray. Infections established from these inoculations provided a source of fresh sphacelial inoculum for further inoculations. In the field inflorescences for experiment were enclosed in paper bags before and after inoculation to prevent cross-contamination with pathogen or pollination, as appropriate. In addition other male-sterile inflorescences were enclosed in insect-proof net (mesh 0.5 × 0.5 mm) 3–5 d before floret gaping, but were not artificially inoculated.

Cryo-preparation and scanning electron microscopy (SEM)

Florets bearing undisturbed white honeydew droplets, 8–9 d post-inoculation, were glued with Tissue-Tek to a copper stub with protective shroud and transferred to the freezing chamber of an EM Scope SP2000 Sputter-Cryo system and frozen in liquid nitrogen pre-cooled *in vacuo* to −210 °C. The honeydew droplet surface was prepared for SEM by fracture in the workchamber with a cooled knife and a macro-manipulator. On the SEM coldstage ice was sublimed and the aqueous surface etched by raising the stub temperature during 4 min to −90° at which it was maintained for a further 3 min. The specimen was gold coated for 4 min (deposition rate 4 nm min^{-1}), and viewed in the SEM (Jeol T200) at acceleration potentials of 2 or 5 kV. Images were recorded on Ilford 120 FP4 film.

Spore-trapping

A Burkard recording volumetric sporetrap sampled continuously for several days at 0.3 m above ground. The air was sampled at a rate of 0·6 m^3 h^{-1} through an orifice 2 × 14 mm and directed at the trapping surface (Melinex tape coated with vaseline/wax; 150 ml vaseline, 18 g paraffin wax, 0·75 g phenol, 500 ml hexane) moving at 2 mm h^{-1}. The tape was cut into 48 mm lengths representing 24 h periods of exposure, mounted on glass slides and deposits stained with 0·1% cotton blue in lactophenol. Vertical transects (588 μm width) were scanned (magnification 250 ×) at 2 mm intervals and the total secondary conidia in each transect counted. Since each transect represents approximately a 15 min deposition period, the concentration of secondary conidia in the sampled air was calculated.

Rotor rods (bearing strips of Melinex tape on the leading edge) operating for 30–40 min were used for qualitative air sampling between 0·66 and 2 m above ground. Tape was stained as above to reveal secondary conidia and other fungal propagules.

Temperature and humidity recording

Measurements at sorghum panicle height were recorded on a shaded thermohygrograph (Casella).

RESULTS

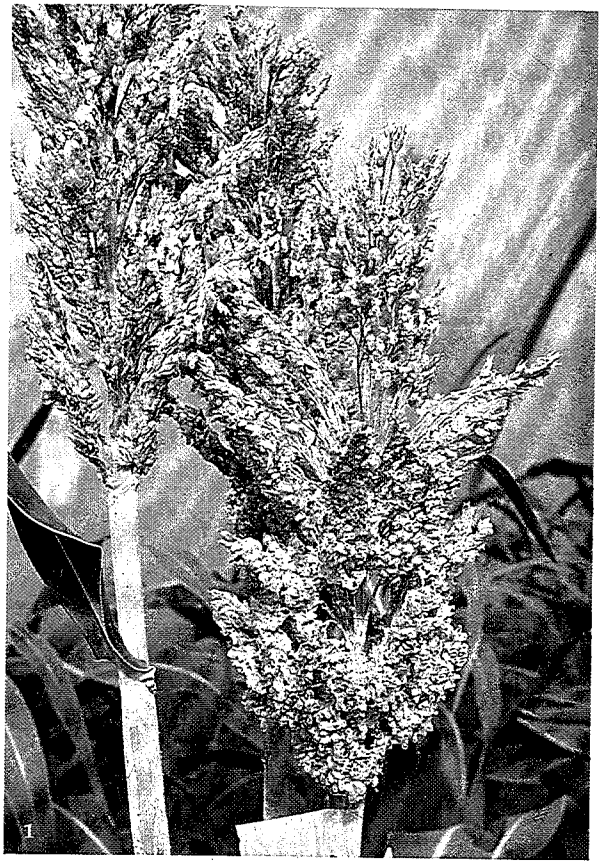
Experimental inoculation of male-sterile sorghum under U.K. horticultural tunnel conditions during Aug. 1987, simulating the range of tropical diurnal temperatures, caused a cascade of honeydew from infected florets. Honeydew was initially colourless and transparent but became progressively opaque on account of sphacelial macroconidia (9–17 × 5–8 μm), together with microconidia (2–3 μm diam), which were swept from the infected ovary in the exudate flow. Within 8–10 d of inoculation infected inflorescences became prominent from a distance of at least 50 m by the whiteness of the exudate surface (Fig. 1).

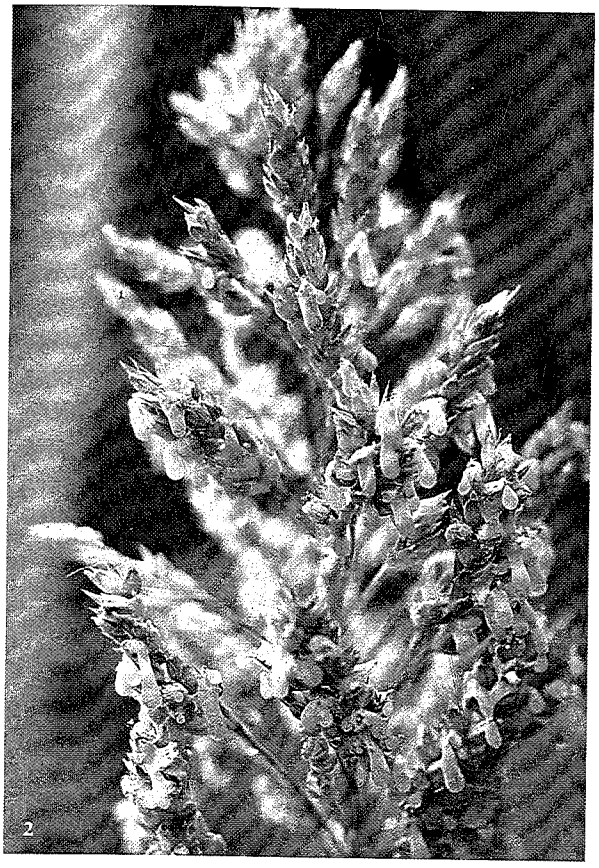
Under conditions of high humidity the exudate consists of enlarged droplets, the new surfaces of which progressively become white (Fig. 2). Cryostage preparations of undisturbed white droplets, freeze-fractured and etched, showed that the whiteness was due to a new surface, raised above the level of the honeydew droplet surface, consisting of a monolayer of spores borne on projecting hyphae which protruded through the droplet surface (Fig. 5). Projecting hyphae originated from the macroconidia immediately beneath the droplet surface and each bore a single secondary conidium (Figs 6–7). Spontaneous ergot infections also occurred subsequently in both male-sterile and fertile sorghum in the same enclosure in the absence of any artificial inoculations and at a distance from sporulating infections which precluded physical contact. Thus, the possibility that new infections had been caused by the secondary conidiation, effectively elevating propagules out of the restrictive confines of a viscous and hypertonic liquid, became apparent.

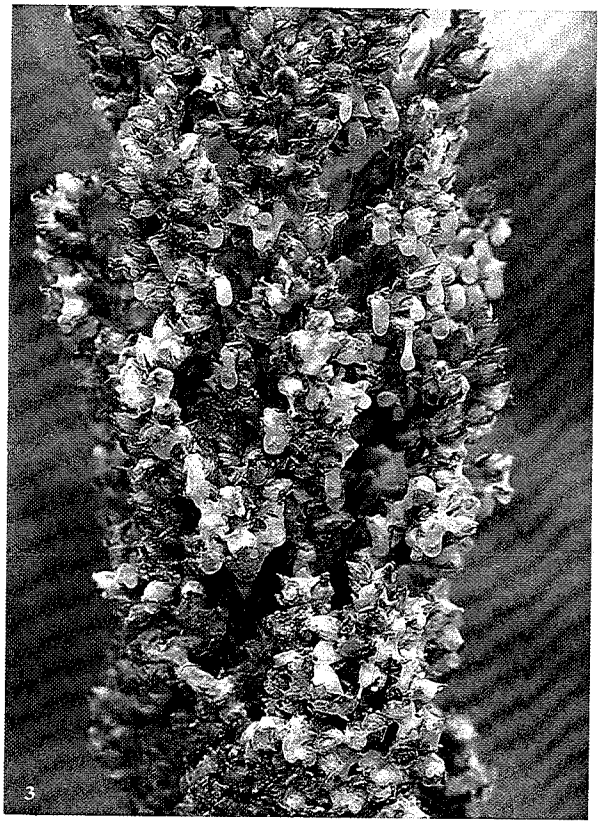
The validity of these observations as expressing a natural phenomenon applicable to field conditions in Southern Africa was tested in Zimbabwe in Mar.–Apr. 1988. Experimental

Figs 1–4. Disease symptoms of extensive ergot (*S. sorghi*) infection of sorghum. Honeydew droplets, initially colourless, develop a layer of secondary conidiation on the surface which, in the case of copious exudation, makes the panicle obtrusively white. **Figs 1–2.** Male-sterile sorghum 8–10 d after experimental inoculation in U.K. conditions. **Fig. 3.** Naturally diseased sorghum in Zimbabwe. **Fig. 4.** Naturally diseased sorghum in Botswana; symptoms are clearly evident at a distance. (See page 499.)

Figs 5–7. Scanning electron micrographs (SEM) of cryostage preparations of honeydew droplets revealing details and derivation of the palisade of secondary conidiation emerging through the liquid surface. **Fig. 5.** Projecting hyphae, arising from superficially located macroconidia and piercing the liquid surface, form an aerial layer on which secondary conidia are borne. Accessory hyphae, a few of which are evident, add to the cohesion of the secondary fructification (Bars = 100 μm). **Figs 6–7.** The fractured liquid surface (right) shows macroconidia, or their impressions, from which projecting hyphae arise (left) immediately below the surface. Projecting hyphae bear single secondary conidia (Bars = 10 μm). Abbreviations; L, honeydew liquid surface; S, projecting hyphae; C, secondary conidia; H, accessory hyphae. **Fig. 8.** SEM of a macroconidium of *S. sorghi* germinating on and penetrating a stigmatic surface of sorghum. The spore had been taken from a honeydew droplet after removing the outer layer of secondary conidiation (Bar = 10 μm). **Fig. 9.** SEM of penetration of a sorghum stigma by the germ tube of a secondary conidium of *S. sorghi*. The spore, the hilum (H) of which is evident, had been wind-borne from infected plants several metres distant (Bar = 10 μm). **Fig. 10.** *S. sorghi* honeydew stained with cotton blue in lactophenol: secondary conidium (S), somewhat pear-shaped and tapered towards the hilar region (H), contrasts with the larger, more oval macroconidium (M) which is often slightly constricted at the centre. Germinated macroconidia with projecting hyphae from which secondary spores have become detached, appear devoid of cytoplasm (Bar = 10 μm). (See page 500.)







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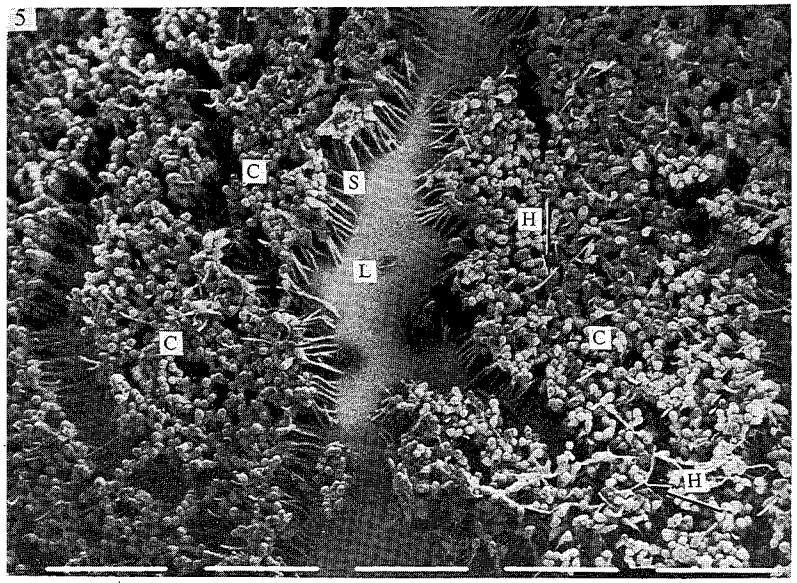
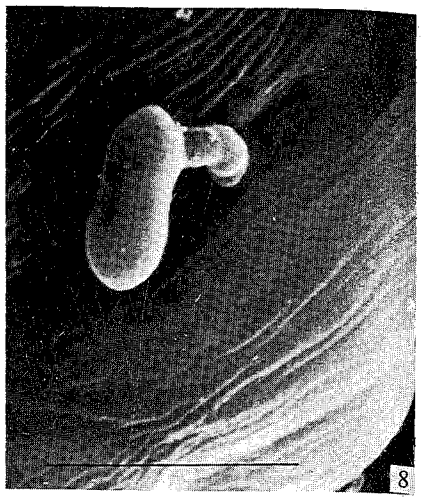
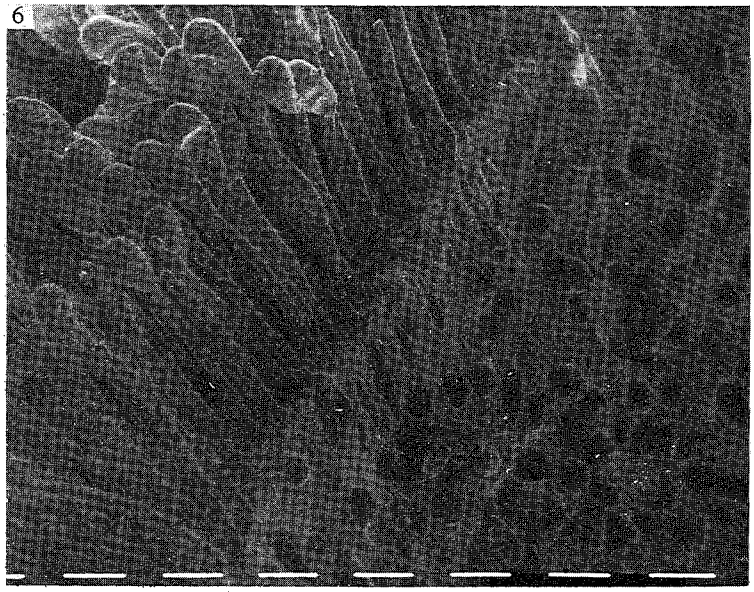
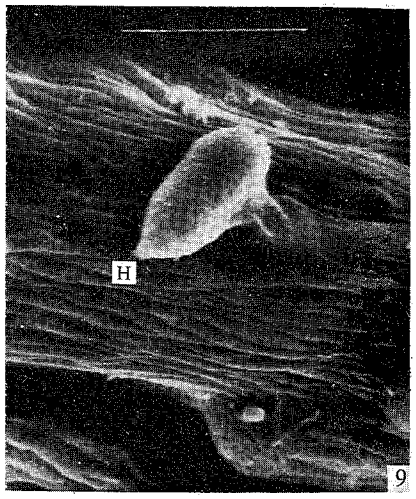
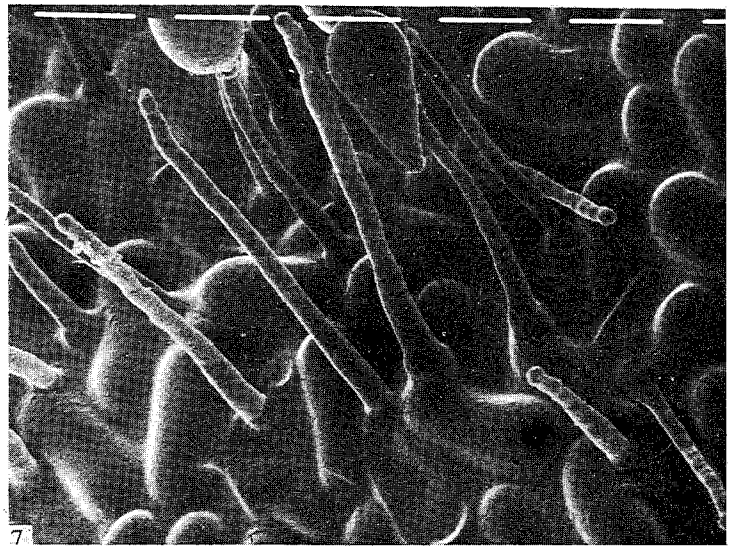
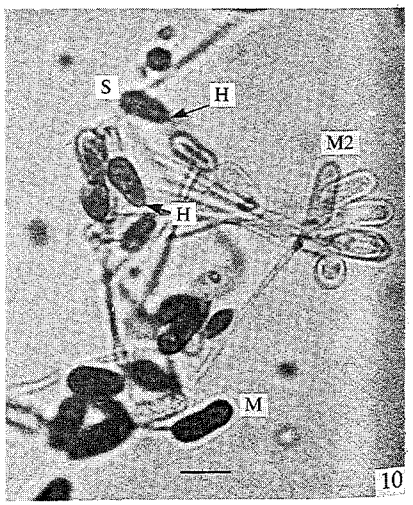






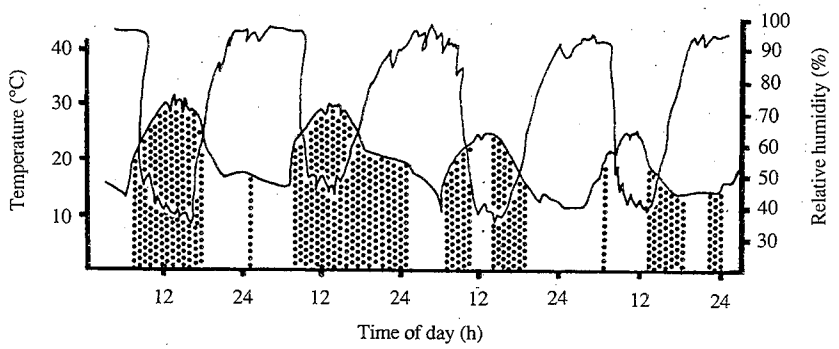






For caption see page 498.

Fig. 11. Dynamics of shade temperature and r.h. during 4 d spore trapping using the Burkard Trap at 0·3 m above ground. Incidence of secondary conidia was determined qualitatively at hourly intervals throughout the period, except on day 3 between 10.00 and 16.00 h when the trap was not operating. Incidence is represented by shading the area under the temperature trace.

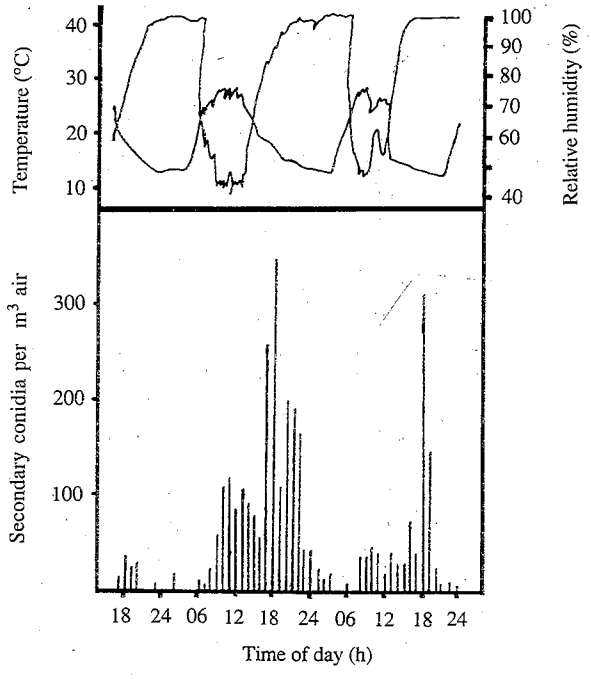


inoculations of male-sterile sorghum, using diluted honeydew, at Bulawayo consistently gave infections which developed the thin white surface of secondary conidia on the honeydew exudate. Natural spontaneous ergot disease at other diverse locations in Zimbabwe (Panmure, Henderson and Aisleby experimental stations) and in Botswana displayed a similar phenomenon (Figs 3, 4). It was consistently evident that the whiteness of ergot-infected inflorescences was due to the secondary conidia and was not just the effect of desiccation and/or crystallization of the honeydew under the intense solar radiation which is one obvious explanation for attributing the term sugary disease (Molefe, 1975).

The superficial white layer of secondary conidia was easily removed, without disturbing the liquid phase of the honeydew, by lightly touching the surface with the tip of a matchstick coated with water agar. The readiness with which they were detached implied a biological function. Microscopic observation of diluted honeydew droplets after removal of some secondary conidia showed germinated macroconidia and their projecting hyphae as characteristic structures mainly devoid of cytoplasm. Each secondary conidium is formed at the total expense of a macroconidium and the slightly smaller size of secondary spores (8–14 × 4–6·5 μm) suggests that external sources of nutrient are not important in the economics of the sporulation, which may therefore be seen as a strategic mycological event to traverse the liquid-gaseous interface and support a potentially airborne propagule. Ungerminated macroconidia deeper in the honeydew droplet still retain the ability to initiate infections by germinating on receptive stigmas and initiating ovary infections in the usual way (Fig. 8). The shape of secondary conidia differs from that of macroconidia and they bear a characteristic hilum marking the point of detachment from the projecting hyphae (Figs 6, 9–10). Secondary conidia are thus readily differentiated from other airspora deposits on sporetrap slides.

Airspora sampling with the rotor rod during the day within an ergot-infected male-sterile sorghum plot revealed the characteristic *S. sorghi* secondary conidia. More detailed continuous monitoring of airspora with the Burkard trap over 2–3 d periods, initially qualitative (Fig. 11) but later quantitative (Fig. 12), showed that the spores were often present during daylight hours but were particularly abundant (*ca* 300 m^{-3}) a few hours after the relative humidity rise associated with nightfall.

Fig. 12. Quantitative incidence of secondary conidia determined hourly over a 2·25 d period, using the Burkard Trap at 0·3 m above ground; the shade temperature and r.h. dynamics are similar to those in Fig. 11.



Natural impaction of airborne secondary conidia on stigmas of male-sterile sorghum, located a few metres from the infective source, and their role as infective propagules was evident from scanning electron microscopy of stigmatic hairs (Fig. 9). Fourteen out of fifteen male-sterile sorghum inflorescences enclosed in insect-proof net and located several metres from ergot-infected plants became infected. In the absence of any vector transmitting inoculum it was concluded that infection had been achieved by airborne propagules passing through the small mesh apertures and impacting in gaping florets.

DISCUSSION

The nearly continuous incidence of secondary conidia in air adjacent to infected plants and the finding that the concentration exceeded 300 conidia m^{-3} at peak periods emphasises that these propagules readily provide inoculum to impact on exposed sorghum gynoecia, thereby initiating disease. It is difficult to quote a very close analogy, but Hirst *et al.* (1955) recorded air concentrations of *Venturia inaequalis* ascospores of an order similar to that found with secondary conidia of *S. sorghi* and deduced that they caused the apple scab disease on newly emergent foliage in two consecutive years. The extent to which *S. sorghi* conidia persist in air and traverse long distances will determine the magnitude of their epidemiological significance.

Microcycle conidiation, iterative germination and secondary conidiation are terms describing analogous processes of spore formation directly from another spore. While many of the expressions of such phenomena occur under particular laboratory conditions, their occurrence in nature may be an important aspect of the biology of filamentous fungi and yeasts. Ascospores of the grass pathogen *Epichloe typhina* (Pers.: Fr.) Tul., closely related to *Claviceps* spp., have recently been shown invariably to germinate directly to give conidia, thus explaining why ascospores themselves fail to initiate infection (Bacon & Hinton, 1988). Deletion of an infective role for a previously-assumed infective *E. typhina* propagule contrasts with the situation in ergot fungi where ascospores are directly pathogenic, but has analogy in *Sphacelia sorghi* where a similar transfer of infective function from one spore to another is apparent. Although transfer of function is at the expense of some macroconidia the creation of an airborne asexual spore partly compensates for the apparent absence of a perennating ascosporogenous phase in the life cycle of this sorghum pathogen. Nevertheless, since no ubiquitous alternate host grass has been identified, there remains a large gap in the rational explanation of the epidemiology of the pathogen particularly with respect to survival between sorghum crops. Ability of macroconidia in dried honeydew on plant debris to survive a period of drought and then to give rise to secondary conidia as rains return would provide the necessary inoculum for renewed infection of the next sorghum crop.

While the present study has demonstrated for the first time that secondary conidiation is important in *S. sorghi* pathology, it is not a phenomenon exclusive to this fungus. In a study of host-parasite relationships in the disease cycle of *Claviceps paspali* Stevens & Hall on *Paspalum distichum* in Georgia U.S.A., Luttrell (1977) observed honeydew in the field with a sparse white layer of phialides projecting above the drops and bearing single conidia. In discussing this feature the possibility that the airborne secondary conidia could cause infection was recognized, but only as a subsidiary means of dissemination. Consequently, the topic was not mentioned in the abstract and at least escaped the present authors' notice until recently. It is probably analogous, however, to the striking phenomenon displayed by *S. sorghi* on sorghum. Whether Luttrell's observations are of a local feature or whether *C. paspali* honeydew generally supports significant secondary conidiation is not clear, but it is surprising that in the extensive annual epiphytotics of ergot on paspalum grasses, particularly *P. distichum* and *P. dilatatum* in, for example, Italy, Australia, New Zealand and South America, secondary conidiation has not been noted. It was not evident in *P. distichum* infections near Bulawayo, Zimbabwe in 1988 and thus contrasted with concurrent observation of typical secondary conidiation of *Claviceps cynodontis* Langdon honeydew on *Cynodon dactylon*. Since secondary conidiation is not exclusive to *S. sorghi* there is no reason to suppose that its expression in *C. cynodontis* implies synonomy. Thus, *C. dactylon* should not be regarded as a potential alternate host for *S. sorghi*. The ergot pathogen (*C. cynodontis*) on *C. dactylon* is a quite different fungus and a distinct species (Loveless, 1965).

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